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Immunisation With Gamma Globulin to Murray Valley Encephalitis Virus and With an Inactivated Japanese Encephalitis Virus Vaccine as Prophylaxis Against Australian Encephalitis: Evaluation in a Mouse Model

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In northwestern Australia, the flavivirus Murray Valley encephalitis (MVE) poses a significant health risk to infants in some aboriginal communities, particularly during each wet season. While there are too few cases to warrant the development of a vaccine against MVE, a safe, effective prophylaxis for these children is still urgently required. The use of passive transfer of human gamma globulin to MVE or immunisation with a vaccine to the closely related Japanese encephalitis (JE) virus were investigated as potential strategies. When 40 μg of IgG was purified from MVE-immune human sera and transferred to 3-week-old mice, the animals were protected from lethal IP inoculation with MVE virus while still producing a detectable immune response to the virus. Similarly, sera from adult mice infected sublethally with MVE or JE virus provided significant protection against MVE infection. However, sera from mice sublethally infected with the related Kunjin or immunised with the inactivated JE vaccine (Biken) provided no protection against MVE challenge. In fact, mice immunised passively with the latter appeared to succumb to MVE challenge more rapidly than mice that received serum from unimmunised animals, suggesting that antibody to the vaccine had accelerated the progression of disease. These preliminary trials in mice indicate that passive immunisation with human gamma globulin has the greatest potential as a strategy for MVE prophylaxis, whilst the apparent enhancement of MVE by antibodies to the JE vaccine requires further investigation, with particular reference to current vaccination programs in areas of Australia and Papua New Guinea, where both JE and MVE occur. ***J. Med. Virol. 61:259–265, 2000.***

KEY WORDS: protection; enhancement; Western Australia

INTRODUCTION

Murray Valley Encephalitis virus (MVE) is a flavivirus that causes a potentially fatal encephalitis in humans. While only 1:200–1:1,000 infected individuals develop encephalitis, clinical cases have an approximately 25% mortality and 50% of survivors have neurological sequelae. The last Australia-wide epidemic occurred in 1974 and was centred on southeastern Australia. Since then human disease has been confined to northern Australia, with 30 cases (7 fatal) from Western Australia, 14 from the Northern Territory, and 4 from northern Queensland. The major mosquito vector of MVE is *Culex annulirostris*, and the dominant animal host is thought to be migrating waterbirds, particularly herons. The virus is maintained in an enzootic cycle in the Northern Territory and in the tropical northeast Kimberley region of Western Australia. People born and raised in these areas are infected almost universally during the first 10 years of life; therefore, clinical disease is seen largely in young children in the enzootic areas, or in people of all ages who have recently entered the region. Epizootic cycles (and occa-

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TABLE I. Details of Virus Strains Used in the Study

Isolate	Virus	Isolation details	Passage history
JE Nakayama	Japanese encephalitis	Japan, 1935	2smb, 1TCVP
MVE F3/51	Murray Valley encephalitis	Mooroopna, Vic., 1951	3TCVP
K 12811	Murray Valley encephalitis	Kimberley, WA 1993	4TCVP
K 6580	Kunjin	Kimberley, WA 1991	4TCVP
35911	Kunjin	Hunter Valley, NSW 1984	6smb 4TCVP
T48	Ross River	Townsville, Qld., 1959	12smb, 3TCVP

Vic., Victoria, Australia: WA, Western Australia, Australia; NSW, New South Wales, Australia; Qld, Queensland, Australia; smb, suckling mouse brain passage; TCVP, tissue culture virus passage.

sional human disease) occur in other areas of the Kimberley as well as the more populated areas of the Pilbara region of Western Australia and in northern Queensland. The infrequent epidemics in southeastern Australia are thought to result from migration of viraemic waterbirds from northwestern Australia when climatic conditions are suitable. When this next occurs, it is expected that there will be large numbers of human cases due to the massive nonimmune population in that area. No vaccines are available for MVE and there is no effective antiviral therapy for flavivirus infections. However, inactivated vaccines are in widespread use that are protective against the closely related Japanese encephalitis virus (JE). In addition, prophylactic passive immunisation with immunoglobulins has been shown to be effective in preventing or ameliorating a number of viral infections, including hepatitis A, hepatitis B, and chickenpox. Weanling mice will develop encephalitis after intraperitoneal injection of MVE or JE viruses, and provide an animal model for evaluation of immunisation. This study uses the mouse model to determine the efficacy of passive immunisation with human anti-MVE immunoglobulin and mouse serum containing JE vaccine induced antibody.

MATERIALS AND METHODS

Virus Strains

Details of the isolation and passage history of the virus strains used in this study are presented in Table I.

Plaque and Neutralisation Assays

Virus titrations were carried out by plaque assay and antibody titres in sera were determined using a plaque reduction neutralisation test (PRNT). Briefly, either virus (plaque assay) or pre-mixed virus and serum samples (PRNT) were inoculated onto low passage number (120–135) Vero cells in 12-well plates (Falcon), incubated for 1 hr at 37°C with 5% CO_2, and overlaid with methylcellulose medium [Hall et al., 1995]. Plates were incubated for several days until plaques were visible by eye. The cells were then stained with methylene blue dye, and the number of plaque forming units (pfu) per well was estimated. The neutralisation titre in the PRNT was estimated as the highest dilution of serum that neutralised 70% of plaques.

ELISA

Human and mouse serum samples were tested for the presence of MVE antibodies by direct enzyme-linked immunosorbent assay (ELISA) as described by Hall and colleagues [1991]. Briefly 96-well microtitre plates were blocked with 0.05 M Tris buffer containing 0.2% w/v casein after coating with either MVE or Ross River virus antigen and incubated overnight at 4°C. Plates were inoculated with samples of human or mouse sera and incubated at room temperature (RT) for 1 hr. Goat anti-mouse or goat anti-human horseradish peroxidase (HRP) conjugate was added and plates incubated for a further hour at RT before adding substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma). Plates were read at the dual wavelengths of 414 and 492 nm using an automated plate reader.

Passive Transfer Experiments in BALB/c Mice, Using Biken Vaccine

The Japanese encephalitis "Biken" vaccine used in these experiments was developed by the Research Foundation for Microbial Diseases of Osaka University, Suita, Osaka, Japan from an inactivated preparation of the Nakayama-NIH strain of JE virus.

A major problem encountered during immunisation/challenge experiments using MVE and JE viruses is that mice older than 3 weeks of age are refractory to peripheral virus challenge. To overcome this, adult mice were immunised and the serum was transferred to 18–20-day-old mice that were susceptible to intraperitoneal (IP) challenge.

Three groups of 40 4-week-old Arc/S mice were inoculated subcutaneously (SC) with 50μl Biken vaccine at three dilutions (neat, 1/10, 1/100) on days 0 and 14, respectively. A fourth group was inoculated sublethally with 100μl of 10^3 pfu live JE Nakayama virus diluted in phosphate-buffered saline (PBS) with 2% foetal bovine serum (FBS) on day 7 by the IP route. Serum from these four groups of mice was harvested on day 28 and used for passive immunisation of groups of 10 18–21-day old BALB/c mice; 250μl of serum was given IP 4 hr before challenge with either 10^3 pfu MVE virus (F3/51) or JE virus (Nakayama). The mice were given a second dose of 250μl antiserum at 16 hr after virus challenge. Mice were observed for up to 21 days and the average time to death estimated for each group. This experiment was repeated using groups of 14 18–20-day-old

mice and challenging with MVE virus only. Statistical significance was determined using the Student's *t*-test comparing the average time to death in each group of vaccinated mice to the control group inoculated with either nonimmune serum or serum positive for Ross River virus antibodies. A P value of <0.05 is considered significant. Outbred mice (Arc/S) were used as donors for antiserum production in an attempt to mimic the variable immune response in humans more closely, while inbred mice (BALB/c) were used as recipients to obtain maximum uniformity of susceptibility to virus challenge for statistical purposes. Weanling (BALB/c) mice were used previously to demonstrate passive protection against MVE challenge by anti-MVE mouse and rabbit antiserum [Hall et al., 1996].

Active Immunisation of BALB/c × C3H/Hej Mice With Biken Vaccine

Three groups of 10 4–5-week-old F1 hybrids were given 50μl of Biken vaccine SC at three dilutions (neat, 1/10, 1/100) on days 0, 14, and 27, respectively. A fourth group of 10 mice were not given vaccine and were used as controls. The mice were challenged intracerebrally (IC) with 5μl of 10^3 pfu MVE virus (F3/51) on day 41 and mortality was monitored for 21 days. These F1 hybrid mice are heterozygous for the flavivirus resistance allele Flv^r and had reduced virus replication in the brain after intracerebral inoculation [Sangster et al., 1994]. When challenged by intracerebral inoculation with approximately 10^3 infectious units of ^{MVE} virus, they show a consistently high mortality rate (70–100%), similar to the genetically susceptible parent strain (BALB/c), but develop encephalitis 2–3 days later, thereby providing more opportunity to demonstrate viral clearance by primed immune responses [Hall et al., 1996]. This mouse model was used previously to demonstrate protection by vaccination with vaccinia and naked DNA constructs expressing MVE viral genes [Hall et al., 1996; Colombage et al., 1998].

Preparation of Human and Murine Immunoglobulin (IgG)

Human IgG. The Kimberley Blood Transfusion Service provided 78 samples of donor serum that were screened for antibodies specific for MVE viruses, using specific monoclonal antibodies in a blocking ELISA described by Hall et al. [1995]. Sera positive for MVE were then confirmed by PRNT against MVE, JE, and Kunjin viruses. Larger samples of MVE-positive sera were then obtained. A total of 600 ml of MVE high-titre (neutralising) serum from three donors was precipitated by the addition of an equal volume of saturated ammonium sulfate and then centrifuged at 10,000*g* for 30 min at 4°C. The pellet was resuspended in PBS and the precipitation procedure repeated twice. The pellet was resuspended finally in Tris buffer (0.02 M, pH 7.2). The γ-globulin was purified by DEAE Affinity-Gel Blue Gel Chromatography (Bio-Rad) and then concentrated using 30K cut-off centricons (Amicon). The centricons were centrifuged at 5,000*g* for 30 min at 4°C and the concentrated protein collected. A sample of nonimmune human serum was also purified in the same way. Protein concentrations were estimated using a Micro-BSA Protein Estimation Kit (Amicon) and MVE antibody titres estimated by PRNT. The results are shown in Table II. The purity of the IgG preparations was confirmed by polyacrylamide gel electrophoresis (PAGE), using the Bio-Rad Protean II apparatus and stained with Coomassie Blue G-250 (Bio-Rad) [Laemmli, 1970].

TABLE II. Properties of Human and Murine Sera Produced in This Study

Purified immunoglobulin	Antibody titre against homologous virus	Total protein (mg)
Human MVE^{+a}	1:160	352
Human MVE^{-a}	<1:20	77.4
Murine MVE[b]	1:6400	7.6
Murine Kunjin[b]	>1:12800	20.2
Murine RRV[b]	1:6400	19.9

MVE, Murray Valley encephalitis; RRV, Ross River virus.
[a]Antibody titre estimated by plaque reduction neutralisation test.
[b]Antibody titre estimated by ELISA.

Murine IgG. Antisera was raised in 4-week-old Arc/S mice by inoculating each mouse with IP 10^4 pfu of either MVE (F3/51), Ross River (T48) or Kunjin (K6580) viruses in 100μl. Sera was collected after 28 days. Pooled sera were then purified and concentrated and protein concentrations estimated in the same way as the human serum. Antibody titres were estimated by direct ELISA (Table II).

Passive Immunisation With Human IgG

In this study, 18–20-day-old BALB/c mice (10 per group) were immunised passively IP with six different doses of human anti MVE IgG (5 mg, 1 mg, 200μg, 40 μg, 8μg, 1.6 μg). Three control groups of 10 mice each were injected similarly with 1 mg, 40μg, and 1.6μg nonspecific human IgG. All groups of mice were challenged IP with 10^3 pfu MVE F3/51 virus and survival monitored for 21 days. Sera was collected by tail bleed from the survivors and tested for murine and human MVE antibody titres by direct ELISA.

Passive Immunisation With Murine IgG

In this study, 18–20-day-old BALB/c mice were immunised passively with four doses (250μg, 10μg, 400 ng, and 16 ng) of MVE, Kunjin, and Ross River virus murine antibodies and challenged with 10^3 infectious units of MVE (F3/51) or 10^4 infectious units Kunjin (35911) viruses. Kunjin strain 35911 was used instead of K6580 for this experiment, as the former is more neurovirulent in mice. Survival was monitored for 21 days after virus challenge.

RESULTS

Cross-neutralisation Activity of Serum Samples From Children Vaccinated Against JE

Pre- and postvaccination sera from 20 children participating in a trial of a new, inactivated JE vaccine in Thailand were kindly supplied by the Armed Forces

TABLE III. Results of Plaque Reduction Neutralisation Tests on Sera from Thai Children Who Were Vaccinated With a Trial Japanese Encephalitis Vaccine

Sample	JE (PRNT 70)[a]	MVE (PRNT 70)[a]
1	20	40
2	40	<20
3	40	<20
4	160	<20
5	40	<20
6	80	40
7	160	40
8	640	40
9	20	<20
10	40	<20
11	40	20
12	80	<20
13	40	<20
14	80	<20
15	80	20
16	40	<20
17	320	40
18	40	<20
19	40	<20
Human anti-JE	320	40
Human anti-MVE	20	160
Human negative	<20	<20

JE, Japanese encephalitis; MVE, Murray Valley encephalitis; PRNT, plaque reduction neutralisation test.
[a]Results expressed as reciprocal of highest dilution of serum that neutralises 70% of plaques.

Research Institute of Medical Sciences (AFRIMS). These were tested by PRNT to determine the neutralisation titres of these sera against MVE (K12811) and JE (Nakayama) viruses. Prevaccination sera from the 19 Thai children inoculated with the JE vaccine had titres of <20 to both viruses by PRNT. All postvaccination sera contained neutralising antibody to JE, with titres ranging from 20 to 640. However, only seven sera neutralised MVE virus with a maximum titre of 40 (Table III).

Cross-Protection Against MVE by Immunisation Against JE

The results of the passive transfer experiments with sera from mice immunised with either Biken vaccine or live JE virus are shown in Table IV. Serum from mice inoculated with neat Biken vaccine provided partial protection to mice against JE virus challenge but no protection against MVE. Sera produced to diluted vaccine (1/10 and 1/100) provided no protection against JE or MVE virus challenge. In addition, it was observed that animals immunised passively with serum from mice inoculated with diluted vaccine died more quickly (approximately 1.5 days) after MVE challenge than those who received nonimmune mouse sera. A repeat experiment confirmed this result showing that mice receiving sera produced to neat and diluted vaccine died approximately 3 days earlier than control mice. In contrast, animals immunised with sera from mice inoculated with live JE virus were protected against both MVE and JE virus challenge.

The results of the active immunisation experiment using F1 hybrid mice showed no significant differences in mortality or time to death in groups immunised with Biken vaccine compared to mice that were not vaccinated (results not shown).

Passive Transfer of Human IgG to MVE

BALB/c mice were immunised passively with six different doses of MVE-reactive human Ig and three doses of nonreactive human Ig. These mice were challenged with MVE virus and survival and time to death recorded. Serum samples were taken from survivors 28 days after challenge and tested by direct ELISA for the presence of both human and murine MVE antibodies. These results are shown in Table V. Doses of anti-MVE IgG higher than 200μg protected animals against MVE infection, but no detectable immune response was produced in the mice. However, when 40μg of IgG was transferred, 90% of the animals were protected from lethal virus challenge while still producing a detectable immune response to the virus. Below this dose there was reduced protection (40–100% mortality).

Passive Transfer of Murine IgG to MVE and KUN

Passive transfer experiments were also carried out to investigate the level of cross-protection provided by murine antibodies raised to MVE and KUN against heterologous virus challenge. The results are shown in Table VI. IgG to MVE virus protected against homologous challenge at a dose of 250μg but not at lower doses. However, mice receiving even the highest doses of IgG raised to KUN virus were not protected against MVE challenge, showing similar mortality rates to those of the control mice that received anti-Ross River IgG (Table VI). Similarly, anti-Kunjin IgG gave 100% protection against homologous virus challenge at doses of ≥10 μg, while no protection against KUN virus challenge was demonstrated with any dose of anti-MVE IgG when compared to Ross River IgG controls. However, the significantly longer time to death seen in mice receiving 250 μg of MVE IgG (14.2 days) as compared with those receiving a similar dose of Ross River IgG (8.5 days) after KUN virus challenge suggest that anti-MVE antibody retarded KUN virus replication in vivo, slowing the onset of disease.

DISCUSSION

The results show that weanling mice immunised passively with murine antisera produced to the Biken JE vaccine were protected against challenge with JE, but not against MVE virus. While it must be noted that these passive transfers did not contain primed B or T cells that may further contribute to protection, the observation that active immunisation with the Biken vaccine also failed to protect against MVE challenge in an alternative mouse model indicates that an inactivated JE vaccine may not provide protection against infection with MVE virus. By contrast, the results of the current study showed that antisera produced by inoculation with live JE virus provides passive protection to mice against lethal challenge with both JE and MVE virus.

TABLE IV. Results of Passive Transfer of Sera from Mice Immunised With Biken Vaccine and Live Japanese Encephalitis Virus

		MVE challenge		JE challenge	
Sera treatment[a]	JE antibody titre by direct ELISA	Average time to death[a]* (days)	Mortality (%)	Average time to death* (days)	Mortality (%)
Arc/S Neat Biken	1:40	8.6 ± 2.3 (P = 0.73)	90	10.8 ± 3.6 (P = 0.15)	40
Repeat experiment		7.1 ± 1.0 (P = 0.001)	100		
Arc/S 1/10 Biken	1:10	7.5 ± 0.9 (P = 0.06)	100	8.8 ± 2.4 (P = 0.15)	90
Repeat experiment		7.1 ± 0.3 (P = 0.001)	86		
Arc/S 1/100 Biken	1:10	7.4 ± 0.8 (P = 0.048)	70	7.3 ± 1.9 (P = 0.95)	100
Repeat experiment		6.8 ± 0.6 (P < 0.001)	93		
Arc/S JE (live)	1:2,560	13.0	10	N/A	0
Nonimmune sera	<1:20	8.9 ± 1.9	100	7.3 ± 1.5	90
Repeat experiment		9.9 ± 2.1	86		

MVE, Murray Valley encephalitis; JE, Japanese encephalitis; ELISA, enzyme-linked immunosorbent assay. N/A, not applicable.
[a]10 mice per group in initial experiment and 14 mice per group in the repeat experiment.
*P-values are calculated by comparing results with nonimmune serum control.

TABLE 5. Results of Passive Immunisation of Mice With Human Ig

Human Ig treatment	Average time to death* (days)		Mortality (%)	Antibodies detected by ELISA (28d)
Anti-MVE 5 mg	N/A		0	Human anti-MVE
Anti-MVE 1 mg	N/A	P < 0.001	0	—
Anti-MVE 200 μg	N/A		0	—
Anti-MVE 40 μg	18	P < 0.001	10	Mouse anti-MVE
Anti-MVE 8 μg	10.5 ± 1.7		40	Mouse anti-MVE
Anti-MVE 1.6 μg	8.5 ± 1.4	P = 0.64	100	—
Nonspecific 1 mg	9.6 ± 1.5		80	—
Nonspecific 40 μg	9.8 ± 2.4		100	—
Nonspecific 1.6 μg	8.1 ± 2.1		90	—

MVE, Murray Valley encephalitis; ELISA, enzyme-linked immunosorbent assay. N/A, not applicable; Ig, immunoglobulin.
*P-values are given only for the dilutions of human MVE Ig that could be compared with the equivalent dilution of nonspecific Ig.

TABLE VI. Results of MVE and Kunjin Challenge of Mice Inoculated With Murine Serum Containing MVE, Kunjin, or Ross River Virus Antibodies

	MVE challenge			Kunjin challenge		
Ig treatment	Average time to death (days)		Mortality (%)	Average time to death (days)		Mortality (%)
MVE 250 μg	N/A	P < 0.001*	0	14.2 ± 1.3	P = <0.001*	50
KUN 250 μg	10.6 ± 2.8	P = 0.112	70	N/A	P = <0.001	0
RRV 250 μg	8.6 ± 1.9		90	8.5 ± 1.8		60
MVE 10 μg	10.3 ± 1.5	P = 0.224	80	8.5 ± 1.7	P = 0.39	100
KUN 10 μg	9.6 ± 1.7	P = 0.68	90	N/A	P < 0.001	0
RRV 10μg	9.2 ± 1.9		100	7.9 ± 1.1		80
MVE 400 ng	8.2 ± 1.9	P = 0.7	90	8.0 ± 2.1	P = 0.88	100
KUN 400 ng	9.9 ± 2.3	P = 0.18	90	9.1 ± 1.5	P = 0.06	70
RRV 400 ng	8.6 ± 1.7		90	7.9 ± 0.9		90
MVE 16 ng	9.4 ± 2.7	P = 0.77	80	7.4 ± 1.1	P = 0.26	90
JYB 16 ng	8.0 ± 1.4	P = 0.29	100	7.8 ± 1.2	P = 0.55	80
RRV 16 ng	9.0 ± 2.4		80	8.1 ± 1.3		90

MVE, Murray Valley encephalitis; KUN, Kunjin virus; RRV, Ross River virus. N/A, not applicable; Ig, immunoglobulin.
*P-values are calculated by comparing results with RRV controls.

This finding appears to correlate with higher levels of antibody produced in mice after infection with JE virus than after inoculation with the inactivated vaccine preparation, which may explain the failure of antiserum from the latter to protect against MVE after passive transfer. The results of the neutralisation assays on the Thai sera also suggest that cross neutralisation against MVE virus challenge only occurs if high levels (>1:80) of JE neutralising antibody are produced after vaccination (Table III). Another factor may have been the generation of antibodies to NS1 by live viral immunisation but not by Biken vaccination (as Biken is purified inactivated virions with little or no NS1 present). Antibody to NS1 has been shown to be protective against experimental infections with flaviviruses [Schlesinger at al., 1985; Gould et al., 1986; Hall et al., 1996; Timofeev et al., 1998] and to play a role in protection against experimental infection with Tick borne

encephalitis (TBE) virus after passive immunisation [Kreil et al., 1998]. Taken together, the results suggest that a live attenuated JE vaccine may have more potential as a prophylaxis against MVE infection. An attenuated vaccine strain of JE virus, SA14-14-2, has been licensed in China since 1988 with 30 million doses distributed annually, but has not yet been approved for use in other countries [reviewed in Tsai et al., 1999].

An unusual but reproducible phenomenon was observed in mice that had been immunised passively with antisera produced to the JE (Biken) vaccine and challenged with MVE virus. The average time to death of mice receiving sera from mice inoculated with Biken vaccine, at dilutions of 1:10 and 1:100, was significantly less than the control group that received nonimmune mouse serum. One possible explanation for this observation is antibody-dependent enhancement, in which the presence of antibody that binds to, but does not neutralise, the infecting virus actually accelerates the progression of disease.

Antibody-dependent enhancement of dengue virus infections has been shown to occur both in vivo and in vitro with low concentrations of polyclonal and monoclonal antibodies to heterologous dengue serotypes [Halstead and O'Rourke, 1977; Halstead, 1982; Morens and Halstead, 1990]. This effect is not restricted to dengue viruses and is thought to be a general property of flavivirus antisera [Peris and Porterfield, 1979; Halstead et al., 1980]. Cross-enhancing activity has been shown to occur among MVE, JE, and West Nile viruses in chick embryo fibroblast cell lines [Hawkes, 1964; Hawkes and Lafferty, 1967] while enhancement of Yellow fever and JE virus infections in mice by monoclonal antibodies reacting to the E protein of these viruses has also been demonstrated [Barrett and Gould, 1986; Gould and Buckley, 1989]. In addition, Kluger et al., [1995] have reported anecdotal evidence of possible enhancement of Tickborne encephalitis infection in humans after passive immunisation with specific Ig. However, these results were not supported by subsequent studies in mice [Kreil and Eibl., 1997].

Halstead and colleagues [1980] suggested that the possibility of enhanced infections resulting from sequential flavivirus infections needs to be evaluated carefully in areas where more than one flavivirus is circulating. This is therefore an important consideration in northern Australia, where MVE and Kunjin and, more recently, JE virus activity has been demonstrated [Broom et al., 1989, 1997; Hanna et al., 1996; Mackenzie et al., 1998]. If JE virus becomes established in northern Australia, the problem of whether to vaccinate the residents of northern Western Australia against JE virus infections would have to be considered. While our preliminary results suggest that low levels of JE antibodies may enhance infection with the indigenous MVE virus, further investigation of this phenomenon is required to elucidate the immune mechanism involved.

In contrast to the above observations, immune mouse serum produced by infection with KUN virus failed to provide passive protection against MVE challenge and vice versa. This may be due to a closer antigenic relationship between MVE and JE, than between MVE and KUN as inferred from earlier cross-neutralisation studies [De Madrid and Porterfield, 1974; Calisher et al., 1989]. In addition, no significant evidence of accelerated time to death was observed in mice after challenge with MVE or KUN when immunised passively with heterologous antibodies at a range of doses. This is an important finding, since people living in areas such as the Kimberley are exposed to both MVE and Kunjin virus infections [Mackenzie et al., 1993].

Our study also explored the possibility of using γ-globulin to protect "at risk" groups (young Aboriginal children and new residents or visitors to the Kimberley) from MVE virus disease at the beginning of the wet season. γ-Globulins have been used successfully to protect against a number of viruses including measles, rubella, hepatitis, and the flavivirus TBE [McDonagh, 1966; Kreil and Eibl, 1997]. Our results show that passive immunisation with doses of $\geq$200 μg of human anti-MVE γ-globulin protect against infection in weanling mice. At these doses of Ig, antibodies to MVE viruses were not produced by recipient mice in response to MVE challenge indicating that infection had been aborted before a detectable antibody response could be mounted. However, mice injected with a lower γ-globulin dose (40 μg) were 90% protected against infection and generated a detectable antibody response to challenge. The ideal situation for using human anti MVE γ-globulins to protect an "at-risk" population would be at a dose of antibody that gave close to 100% protection against infection but also allowed priming of the immune response to provide long term immunity against subsequent infections with MVE virus. Clinical trials will be required to determine the appropriate dose of γ-globulin to achieve this balance in human recipients.

STATEMENT

It should be noted that death as an endpoint was avoided in accordance with the Australian Code of Practice. All animal experiments were approved by the Animal Experimentation Ethics Committee at the University of Western Australia and all workers had current animal experimentation licenses.

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